Marquis

A Study Of The Amino-Acid Content Of Blood Meal.

A STUDY OF THE AMINO-ACID CONTENT OF BLOOD MEAL

BY

STEWART DENT MARQUIS

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Stewart Dent Marquis

ENTITLED A Study of the Amino-Acid Content of Blood Meal

BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science in Chemistry

H. S. Grindley
In Charge of Thesis

W. A. Noyes
Head of Department

Recommendation concurred in:*

Committee
on
Final Examination*

*Required for doctor's degree but not for master's.

THE AMINO-ACID CONTENT OF BLOOD MEAL

INTRODUCTION

Amino-Acids as Constituents of Proteins

For many years it has been known to chemists that the proteins, those complex nitrogen-containing bodies of living tissue, are made up primarily of a group of compounds called the amino-acids. It was in 1820 that Braconnot[1] obtained glycocoll by boiling gelatin and meat with sulfuric acid, and gave the name "leucin" to an acid which Proust[2] had discovered as a decomposition product of cheese. In 1849[3] Liebig obtained tyrosin from hair, in 1865[4] Cramer found serine in silk, and in 1867[5] Kühne discovered both leucine and tyrosine among the products of the tryptic digestion of fibrin. During the next twenty-five years an ever-increasing number of investigators substantiated these results and added, one by one; to the list of amino-acid substances derived from proteins. In 1891, Drechsel[6] gave new impetus to the work by his identification of one of the hexone bases, lysine, among the hydrolysis products of albumin. This was quickly followed by Hedin's[7] separation of arginine from the same source, and Kossel's[8] discovery of histidin. Today, the number of laborers in this field is legion, and proteins from every source have been resolved into their constituent "Bausteine", or "building stones".

The methods used in the cleavage of the proteins have been many, but fall mainly into three classes, namely:

(1) Hydrolysis by acids

(2) Hydrolysis by alkalis

(3) Hydrolysis through the agency of enzymes, derived from living tissue.

It is in the last of these three methods that students of nutrition are most closely interested, for it reproduces most nearly the conditions which

obtain in the animal body.

The Importance of Amino-Acids in Animal Nutrition

The researches of Bergmann,[9] Folin and Denis,[10] and many others have shown unquestionably that the proteins of ingested food are not carried as such to the tissues, but that they are first split by the enzymes of stomach and intestines into amino-acids, which are then resynthesized at some point in the circulatory system, to form the tissue proteins. The exact place and method of synthesis are questions for dispute, but it is clearly established that the proteins are reduced to their amino-acids, then rebuilt, before being made into tissue. With this fact proven, two questions naturally arise (1) Does every protein ingested in the food contain all of the amino-acids required by body-proteins? (2) Can the amino-acids replace one another as protein-builders, or has each a specific function in that respect?

The protein analyses of Abderhalden, of Kossel and Kutscher, of Fischer, of Van Slyke, and many others, have proven that certain proteins are lacking in individuals of the characteristic amino-acids found in most proteins. Some of these are

Gelatin, which contains no tryptophané, tyrosine or cystine

Zein, which contains no lysine, tryptophane or glycocoll

Gliadin, which contains no lysine.

With regard to the second question, the classic researches of Osborne and Mendel[11] and of McCollum and Hart[12] have given at least the beginnings of an answer. Osborne and Mendel, with albino rats as subjects, studied the effect of dietaries containing as the sole protein constituent a protein lacking in one or more of the amino-acids. They then added the missing acids to the ration and recorded the effects. With pigs as subjects, McCollum and Hart carried out somewhat similar investigations. The results of their experiments, while conflicting in some respects, agreed in the main, and have

thrown new light on the subject of the roles played by individual amino-acids in animal-maintenance and growth. These results may be summarized in the following general considerations:

(1) Certain amino acids, particularly glycocoll, can be synthesized by the animal body, hence are not indispensable constituents of the food.

(2) Certain amino-acids must be provided in the food to insure maintenance of the animal - that is, continued life at practically constant body-weight, with no signs of growth. Tryptophane is the only one of this class whose identity is clearly established.

(3) Certain amino-acids are indispensable constituents of the diet, if growth as well as maintenance, is desired. Of the group, lysine is the example.

(4) There is a minimum of each of these indispensable "Bausteine" which must be provided in the diet of proper maintenance and growth are to be secured. This minimum varies probably with individual species and perhaps with individuals of each species of animal.

(5) It may be mentioned as interesting and possibly confirmatory of the experiments quoted above that, according to the best available analyses, such proteins as ovalbumin, lactalbumin, and casein, which are common constituents of the food of both man and beast, contain relatively large amounts of lysine and tryptophane.

The work of these investigators has opened up a field of labor, the importance of which can scarcely be overestimated. To quote Osborne and Mendel,[13] "The current trend of the investigation of the Chemistry of Nutrition is emphasizing the significance of the amino-acids as the fundamental factors in the problems in which hitherto the role of the proteins has been involved. The remarkable success which has attended the efforts to supplant completely the proteins in the food intake by their ultimate products of hydrolysis - the so-called "Bausteine" - has led to promising researches in which these food fragments

have been followed beyond the alimentary barrier into the blood stream to the tissues, and almost to their final destruction in the body. The question of protein synthesis now becomes a problem of the biochemical department of the amino-acids."

The possibilities which lie ahead in this field are enormous, but it must be admitted that too far-reaching interpretations cannot as yet be put upon the results. Studies on the nutrition of albino rats, while they undoubtedly furnish illuminating evidence and point the way to further investigation, cannot be considered as entirely analogous to nutritive experiment, conducted with other animals as subjects. The practical value of the results gained will depend very largely on further tests in which horses, cattle, sheep and other farm animals are used. Many difficulties block the path of the investigator. Pure proteins are very difficult to obtain, and the desired amino-acids even more so. Another problem which presents itself is the difficulty of making the ration attractive to the animal, so that it will all be consumed. But these and many other problems are being gradually overcome, and new investigations are underway in various laboratories. These investigations will undoubtedly bring to light more data concerning the specific functions of the amino-acids and hence will indicate the relative nutrient value of the proteins of which they are components.

The Amino-Acid Content of Feeding-Stuffs.

Investigations as to the nutritive value of isolated proteins and the role of individual amino-acids in nutrition are important to the physiologist and physiological chemist, in that they furnish evidence as to the final fate of these substances in the body. But to the student of animal nutrition and to the farmer himself, these studies will have value, only as their results may be applied to the conditions under which he labors. As McCollum says, "Studies relating to the values of isolated proteins are of fundamental import-

ance in revealing the character of the chemical processes involved in nutrition, but practical dietetics and animal production must always deal with certain groups of proteins as they are found in the naturally occurring foodstuffs.[14] Zein, for instance, while one of the important proteins of corn, is not the only protein contained therein, and it may well be that the tryptophane and lysine necessary for maintenance and growth are found in these other constituents of the kernel. If, therefore, the true nutritive value of a feeding-stuff is to be determined, the identity of the proteins which it contains must be established, and their content of amino-acids estimated. Furthermore, the possible effect of one protein upon another, when they are mixed in varying proportions, and of the other constituents of the food, such as carbohydrates and fats, must be thoroughly understood. In the light of these considerations, the enormity of the problem and the danger of deduction from insufficient results become apparent.

If the trend of recent investigation is to be believed, however, it is to their ultimate amino-acid content, rather than to the proteins as such that feeding stuffs owe their specific value. It would seem of fundamental importance then, that the amino-acid content of the various foods be estimated, and two possible methods for such estimation present themselves.

1. The proteins may be isolated from the feedingstuff, and their amino-acid content determined, if not already available. Such a scheme presents the obvious advantage, that after the pure proteins have been obtained, their amino-acids can be estimated without danger of interference by other compounds. The disadvantage of the method is that the isolation of the proteins is a long and tedious process, and that the methods now known are not quantitative. Furthermore, this method would not detect the small quantity of free amino-acids which are always present in feeding-stuffs.

2. The feeding-stuff may be hydrolyzed direct, and the resulting amino-acids estimated. This method has the advantages of comparative speed, and will account for all of the amino-acids both free and combined present in the feeding-stuff. Its chief disadvantage lies in the possible sources of error through incomplete hydrolysis or further decomposition of the products through the influence of the non-protein substances present.

On the whole, the latter method seems preferable, particularly for the analysis of blood meal, which contains almost 90 percent of protein and only 10 percent of non-protein substances. For the other feeding-stuffs this method is also more practicable until methods of protein isolation are perfected. Any interpretation of results must, of course, be subject to correction by later investigation which may reveal a plan for eliminating the possibilities of error.

The Amino-Acids of Proteins

Up to the present time, seventeen amino-acids have been identified as common hydrolysis products of the proteins, and several others have been described in isolated instances. These seventeen are as follows:

A. Mono-amino-mono-carboxylic acids

1. Glycine, $C_2H_5NO_2$, glycocoll, or amino-acetic acid

$CH_2(NH_2).COOH$

2. Alanine, $C_3H_7NO_2$, or alpha-amino-propionic acid

$CH_3.CH(NH_2).COOH$

3. Valine, $C_3H_{11}NO_2$, or alpha-amino-isovalerianic acid

$$(CH_3)_2CH.CH(NH_2).COOH$$

4. Serine, $C_3H_7NO_3$, or alpha-amino-beta-hydroxy-propionic acid

$CH_2(OH)CH(NH_2).COOH$

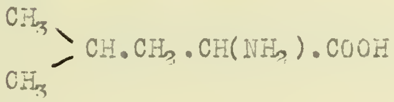
5. Phenylalanine, $C_9H_{11}NO_2$, or beta-phenyl-alpha-amino-propionic acid

$C_6H_5.CH_2.CH(NH_2).COOH$

6. Tyrosine, $C_9H_{11}NO_3$, or para-oxy-beta-phenyl-alpha-amino-propionic acid

$$HO.C_6H_4.CH_2.CH(NH_2).COOH$$

7. Leucine, $C_6H_{13}NO_2$, or alpha-amino-iso-butyl acetic acid



$$\begin{matrix} CH_3 \searrow \\ \\ CH_3 \nearrow \end{matrix} CH.CH_2.CH(NH_2).COOH$$

8. Isoleucine, $C_6H_{13}NO_2$, or alpha-amino-beta-methyl-beta-ethyl-propionic acid

$$\begin{matrix} CH_3 \searrow \\ \\ C_2H_5 \nearrow \end{matrix} CH.CH(NH_2).COOH$$

9. Cystine, $C_6H_{12}O_4N_2S_2$, or alpha-diamino-beta-dithiolactyl acid

$$HOOC.CH(NH_2).CH_2S-S.CH_2.CH(NH_2).COOH$$

B. Mono-amino-di-carboxylic acids

10. Aspartic acid, $C_4H_7NO_2$, or amino-succinic acid

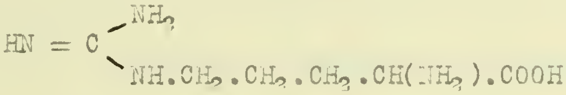
$$HOOC.CH_2.CH(NH_2).COOH$$

11. Glutamic acid, $C_5H_9NO_4$, or alpha-amino-normal-glutaric acid

$$HOOC.CH_2.CH_2.CH(NH_2).COOH$$

C. Di-amino-mono carboxylic acids

12. Arginine, $C_6H_{14}N_4O_2$, or guanidine-alpha-amino-valerianic acid.



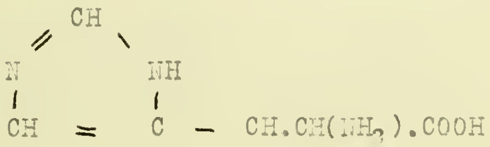
$$HN = C \begin{matrix} \nearrow NH_2 \\ \\ \searrow NH.CH_2.CH_2.CH_2.CH(NH_2).COOH \end{matrix}$$

13. Lysine, $C_6H_{14}N_2O_2$, or alpha-epsilon-diamino-caproic acid

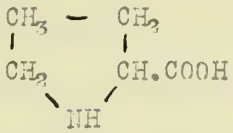
$$H_2N.CH_2.CH_2.CH_2CH_2.CH(NH_2).COOH$$

D. Heterocyclic Compounds

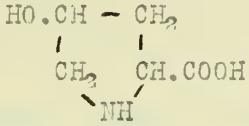
14. Histidine, $C_6H_9N_3O_2$, or alpha-amino-beta-imidazol-propionic acid



15. Proline, $C_5H_9NO_2$, or alpha-pyrollidine-carboxylic acid.

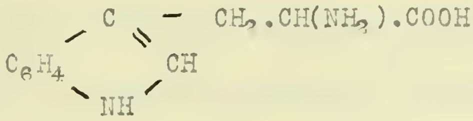


16. Oxyproline, $C_5H_9NO_3$, or oxypyrollidine carboxylic acid



(the position of the -OH group is doubtful)

17. Tryptophane, $C_{11}H_{12}N_2O_2$, or indol-alpha-amino-propionic acid



The constitution of those less common is not definitely established, and at present their importance may be regarded as negligible.

It is by no means certain that amino-acids now unknown will not be discovered among the products of protein hydrolysis. It can only be said, that with the methods of protein analysis at present available, those enumerated have been isolated, but there is a strong probability that others will be found.

Knowledge of the method of union of the amino-acid unity to make up the protein molecule is at present very limited, although the synthesis of the so-called polypeptides by Fischer, Abderhalden and others has given valuable information on that question. It is known, however, that the protein molecule is very large - some estimate the molecular weights as high as 25,000. The nature of the process of cleavage by acids or enzymes remains obscure at present, although it seems probable that the amino-acid units are split off one by one till the whole molecule is decomposed.

The Determination of Amino-Acids in Proteins

The problem of the identification of the products of protein hydrolysis has engaged the attention of investigators since amino-acids were first discovered. A detailed account of the methods which have been proposed for the detection, both qualitatively and quantitatively of the individual amino-acids, is not within the scope of this paper. Suffice it to say that, although an immense amount of labor has been expended, the results are still far from satisfactory.

Four methods of determination are in use at the present time.

1. E. Fischer's esterification method for the estimation of the monamino acids. By this method, first proposed by Fischer in 1901,[13] and since developed by himself and others, the monamino acids are converted into their respective Ethyl esters, which are then separated by fractional distillation under a very low pressure. The esters are then reconverted to the acids by boiling with water or barium hydrate.

A large number of proteins have been analyzed by this method, in combination with the Kossel-Kutscher method described below, but invariably a considerable percentage of the nitrogen has not been accounted for. As Abderhalden says,[14] "In the isolation of individual dissociation products, losses are unavoidable: to obtain amino-acids quantitatively is impossible even after a triple esterification, for the residue still possesses a strong smell of esters, after the last ether extraction." Such values as have been obtained by this method must be considered as minimal.

2. The β-naphthalin sulphonate method, proposed in 1902 by Fischer and Bergell.[15] The monamino acids are converted into β-naphthalin-sulpho-derivatives, which are but slightly soluble substances, and these are separated by fractional crystallization. Fischer, Bergell and Abderhalden have used this method on a variety of proteins, but here again the values obtained are only minimal.

3. The Kossel-Kutscher method for estimation of the hexone bases, lysine, arginine and histidine, as proposed in 1900[16], and since perfected. By treatment with silver sulfate and saturation with barium hydrate, histidin and arginine are precipitated. The precipitates are dissolved in acid, the barium and silver removed, and the histidine and arginine separated by fractional precipitation with fresh portions of silver nitrate and barium hydrate. The lysine is precipitated from the original filtrate with phosphotungstic acid, and converted into the picrate for determination. If care is observed, the determination is quantitative, and excellent results have been obtained with it by Kossel and Kutscher, Abdernalden, Hart, and others.

4. The nitrogen distribution method of Van Slyke, based on the plan first proposed by Hausmann.[17] Van Slyke first published the details of the method in 1911.[18] He does not attempt to isolate the acids, but his analysis divides the nitrogen into groups, whose quantity can be accurately determined. He makes use of the well-known reaction of amino-nitrogen with nitrous acid, whereby nitrogen is evolved.

$$R.NH_2 + HNO_2 = ROH + H_2O + N_2$$

The groups into which the nitrogen of the protein molecule is divided are as follows:

(1) Amide N.
(2) Humin or melanin N.
(3) Arginine N
(4) Cystine N.
(5) Histidine N.
(6) Lysine N.
precipitated by phosphotungstic acid
(7) Amino N unprecipitated by phosphotungstic acid
(this includes all the N of glycine, alanine, valine, leucine, iso-

leucine, phenylalanine, tyrosine, serine, aspartic acid, glutamic acid, and one-half the N of tryptophane, with the possible addition of monamino acids as yet undiscovered).

(8) Non-amino N unprecipitated by phosphotungstic acid.

(includes all the N of proline and oxyproline, and one-half the N of tryptophane).

It will readily be seen from the above outline that as yet no thoroughly satisfactory method for the estimation of all the products of protein hydrolysis has been proposed. The determination of histidine, lysine, and arginine seems to be fairly well established, as the Kossel-Kutscher and Van Slyke methods give results which agree within reasonable limits of error. The non-amino acids remain the great problem, and it will be apparent from the table given below just how far the Fischer method has fallen short of quantitative results.

On the whole, the Van Slyke method gives the best insight into the composition of the protein molecule. It possesses two distinct advantages in manipulation over the combination of the Kossel-Kutscher and Fischer methods, namely, speed and comparative size of sample required. A complete Van Slyke analysis may be made in a few days, while the esterification requires two to three weeks, even in the hands of a skilled manipulator. A sample containing 3-4 grams of protein gives satisfactory results by the Van Slyke method whereas 25-30 grams must be used for the Kossel-Kutscher analysis and 250-500 grams for the Fischer esterification.

Van Slyke and others have analyzed a number of pure proteins and protein mixtures with excellent results. Some of these analyses are given in the table below, together with analyses of the same proteins by the Fischer and Kossel-Kutscher methods.

TABLE I. A COMPARISON OF PROTEIN ANALYSES

(Figures represent percentage of total N)

Protein	Amide N		Humin N		Arginine N		Cystine N		Histidine N		Lysine N		Amino N of filtrate		Non-amino N of filtrate	
	Van Slyke	Others	Van Slyke	Others	Van Slyke	Others	Van Slyke	Others	Van Slyke	Others	Van Slyke	Others	Van Slyke	Others	Van Slyke	Others
Gelatin	2.25	2.2	.07		14.0		0		3.59		6.20		57.0	27.0	16.0	9.5
Edestin	9.99		1.98		27.05	25.0	1.49	1.60	5.75	3.5	3.86	1.8	47.55	20.7	1.7	0.7
Gliadin	24.61	25.2	0.58		5.45	6.44	0.80		3.39	2.7	1.33	0	51.95	29.9	10.7	5.1

Two things are apparent from the above table:

(1) The largest differences between the Van Slyke and other methods occur in the non-amino N (amino N of filtrate). This is to be expected because of the inevitable losses involved in analysis by the esterification method.

(2) Proteins differ markedly in their amino-acid make-up, and hence differences are to be expected in their nutritive value.

Determination of the Amino-Acids of Feeding-Stuffs

As pointed out above, the determination of the amino-acid content of feeding-stuffs themselves, rather than that of pure proteins, must always be of the greater value to the practical animal husbandry man. Some of the possibilities opened up by such studies are pointed out by Grindley, Joseph and Slater.[19]

"The quantitative determination of the amino-acids of feeding-stuffs together with the aid of the rapidly increasing evidence on the nutritive functions of the amino-acids will make it possible to extend our present knowledge of feeding-stuffs along the following lines:

"(1) The results will make it possible to calculate balanced rations from the standpoint of their amino-acid content that will be most efficient for the maintenance, growth and the fattening of farm animals.

"(2) The results will be helpful in the interpretation of the results of past and future feeding experiments.

"(3) The results should lead to a method of applying the fast accumulating results, as to the nutritive value of amino-acids, to the economic and nutritive value of the common feeding-stuffs.

"(4) The results will make it possible to plan and to make feeding experiments with farm animals with the natural mixtures of proteins as they occur in common feeding-stuffs, that will aid in the determination of the relative efficiency of the proteins of different feeding-stuffs for the maintenance, the growth and the fattening of farm animals."

Grindley and his coworkers were the first to undertake the direct determination of the amino-acids of feeding-stuffs, and their published results[20] furnish some very interesting data. Analyses of the same feedingstuffs since published by Nollau[31] do not give always concordant results for similar feeding-stuffs, but this lack of agreement seems to be due to dissimilarity of samples and to slight differences in procedures used. It must be recognized, however, as pointed out before, that the non-protein substances of the feeding-stuff constitute a possible source of interference with the accuracy of the determinations. Succeeding experiments should throw light on this point. In any case, such determinations undoubtedly give approximately quantitative results, whose value is sufficient to warrant use of the method in the determination of the amino-acids of feeding-stuffs, till a better method is developed.

EXPERIMENTAL PART

With a view to obtaining further information as to the availability of the Van Slyke method for determining the amino-acids of feeding-stuffs, samples of blood meal were subjected to analysis.

Outline of the Method Used

The method of analysis as originally recommended by Van Slyke[18] was used throughout, with slight alterations deemed necessary for the purposes of this analysis.

The Hydrolysis

A representative sample of blood meal was ground to a fine powder, and three samples of approximately eight grams each weighed out for analysis. Each of these was transferred to a round bottom flask connected with a Hopkins reflux condenser. 200 cc. of 20 percent HCl was added and the mixture heated over a boiling water-bath for four hours. A free flame was then substituted for the water bath, and the mixture was boiled for sixteen hours, with frequent shaking

to insure hydrolysis of all portions of the sample. At the end of this period the boiling was stopped, the solution cooled and two 1 cc. portions removed. These were diluted to 10 cc. and the amino-nitrogen determined in the Van Slyke apparatus. The hydrolysis was then continued for four hours, when a determination of the amino-nitrogen showed the hydrolysis to be complete. The customary precaution was taken of weighing the flask and contents before the hydrolysis and at each stopping point to correct for any increase in concentration through loss of vapor.

Determination of Total Nitrogen

After removal of excess of hydrochloric acid by concentration under reduced pressure, the mixture was made up to 250 cc. and portions of 10 cc. taken in triplicate for total nitrogen determination by the Kjeldahl method.

Determination of Ammonia (Amid Nitrogen)

Of the remaining mixture, two portions of 100 cc. each were taken for determination of ammonia nitrogen. Each was treated with Ethyl alcohol to prevent foaming, then an excess of a 10 percent suspension of calcium hydrate added, and the ammonia distilled under reduced pressure into tenth normal HCl. The excess HCl was titrated with tenth normal alkali and the ammonia calculated.

Determination of Melanin (Humin) Nitrogen

The mixture remaining from the ammonia determination was filtered, the residue thoroughly washed, and the total nitrogen of the remaining solid determined by the Kjeldahl method. The result was taken as the measure of the melanin nitrogen.

Precipitation, Washing and Redissolving of the Bases

After concentration in vacuo of the filtrate from the melanine determination the bases were precipitated from the solution with excess of phosphotungstic acid. It was necessary to add about 21 gms. phosphotungstic acid to secure an excess. The mixture was heated for some time in a steam bath, then allowed

to stand forty-eight hours to insure complete precipitation. It was then filtered through a Buchner funnel fitted with a hardened filter and the precipitate washed with about 100 cc. of the wash solution of 2.5 percent phosphotungstic acid and 3.5 percent hydrochloric acid. The precipitate was then dissolved from the filter with 20 percent sodium hydrate. The solution was diluted to 800 cc. and barium chloride added in sufficient excess to precipitate the phosphotungstic acid. The precipitate was filtered off and the solution concentrated in vacuo to a small volume. Because of the high protein content of the blood-meal, and consequent excess of amino-acids in the solution at this point, it was diluted to 100 cc. rather than 50 cc. as recommended by Van Slyke, thereby making it possible to make duplicate determinations for total and amino nitrogen of the bases.

Determination of Arginine

Arginine was determined in 25 cc. of the solution of the bases by boiling for six hours with potassium hydrate. The ammonia was collected in tenth normal HCl in Folin bulbs, and the excess HCl titrated with NaOH. The arginine nitrogen was calculated as usual from the ammonia evolved.

Determination of Total Nitrogen of the Bases

Duplicate 10 cc. portions of the solution of the bases were used to determine total N of the bases by the Kjeldahl method.

Determination of Cystine N

Duplicate 10 cc. portions of the solution of the bases were used to determine cystine N, by oxidation of the sulfur with $Cu(NO_3)_2$ precipitation as $BaSO_4$, ignition and weighing.

Determination of the Amino-Nitrogen of the Bases

Duplicate 10 cc. portions of the solution of the bases were used to determine the amino-N by the Van Slyke apparatus.

Calculation of the Histidine-N and Lysine-N

The histidine nitrogen was calculated by Van Slyke's method, dependent upon the fact that arginine contains three-fourths of its nitrogen and histidin two-thirds of its nitrogen in the non-amino form, while all of the nitrogen of lysine and cystine is in the amino form. If, then, three-fourths of the arginine nitrogen be subtracted from the total non-amino nitrogen of the bases, the result multiplied by three halves gives the histidine nitrogen.

The lysine nitrogen is then obtained by difference: Lysine N = Total N of bases - (Arginine N + Histidine N + Cystine N). The usual corrections were made for solubilities of the phosphotungstates of the bases.

Examination of the Filtrate from the Bases.

The filtrate from the original precipitation of the bases with phosphotungstic acid, was made neutral with NaOH, concentrated in vacuo, and made up to 250 cc., rather than 150 cc. because of the large quantity of nitrogen present.

The total nitrogen of the filtrate was determined in duplicate 10 cc. portions of the solution by the Kjeldahl method.

The amino-nitrogen of the filtrate was estimated in duplicate 10 cc. portions in the Van Slyke apparatus.

The non-amino nitrogen of the filtrate was then obtained by difference.

Reagent and Apparatus Used.

Blank determinations were made of the N in the reagents for Kjeldahl analysis, and in the sodium nitrite solution and in the amino-N determinations. Calibrated apparatus was used for measuring all volumes.

Results of Analyses

The analytical results are shown in Tables II and III, which are self-explanatory. Table IV shows some of the results obtained by Grindley and his coworkers[24] and is appended for comparison.

TABLE II. AMINO-ACID CONTENT OF BLOOD MEAL

(Expressed in percentage of total nitrogen)

Sample	Amide N	Humin N	Arginine N	Cystine N	Histidine N	Lysine N	Amino N in filtrate from bases	Non-amino N in filtrate from bases	Total N by summation
I	5.98	3.90	9.47	0.70	8.45	9.89	55.21	4.73	98.33
II	5.74	3.81	8.97	0.68	8.52	9.59	57.29	4.32	98.92
III	5.83	3.83	9.04	0.68	8.63	9.70	57.20	4.21	99.12
Average	5.85	3.85	9.16	0.69	8.53	9.73	56.57	4.42	98.80

TABLE III. AMINO-ACID CONTENT OF BLOOD MEAL

(Expressed in percentage of the feed)

Sample	Amide N	Humin N	Arginine N	Cystine N	Histidine N	Lysine N	Amino N in filtrate from bases	Non-amino-N in filtrate from bases	Total N by summation	Total N by analyses
I	0.837	0.546	1.325	0.098	1.041	1.383	7.724	0.662	13.616	13.988
II	0.803	0.534	1.255	0.095	1.191	1.342	7.907	0.604	13.731	13.988
III	0.815	0.536	1.264	0.095	1.305	1.356	7.982	0.589	13.942	13.988
Average	0.818	0.539	1.281	0.096	1.179	1.360	7.871	0.618	13.762	13.988

TABLE IV. NITROGEN OF THE AMINO-ACIDS OF FEEDING-STUFFS

(Results expressed in percentage of total nitrogen of feeding-stuff)

Feeding-stuff	Amide N	Humin N	Arginine N	Cystine N	Histidine N	Lysine N	Amino N in filtrate from bases	Non-amino N in filtrate from bases	Total N by summation
Tankage	6.58	4.40	14.15	1.28	4.94	7.48	52.39	7.27	98.49
Wheat	17.59	9.21	7.99	1.34	1.67	2.47	47.67	13.59	101.53
Oats	13.06	9.94	11.42	1.16	4.32	3.49	51.72	7.90	103.01
Cottonseed Meal	10.45	7.78	19.52	0.65	5.47	4.78	42.82	5.43	96.90
Alfalfa Hay	8.44	15.79	7.68	0.88	7.44	4.10	44.02	9.79	98.14

By a comparison of Tables II and IV above, several interesting things are apparent.

1. The lysine content of blood-meal is higher than that of any of the other feeding-stuffs, the difference being from 2.25 percent to 7.26 percent. If Osborne and Mendel's conclusion be true, that lysine is necessary for growth, the well-known value of blood meal as a supplemental stock food would be explained.

2. A summation of the total amino-nitrogen of blood meal gives 89.1 percent of the total nitrogen, a higher figure than for any other of the feeds tested, tankage being next with 87.54 percent. This might confirm the conclusion deduced from the lysine content.

3. There is less humin nitrogen in blood meal than in any of the other feeds, the difference ranging from 0.5 percent to 11.89 percent.

By a comparison of Tables II and IV with Table I, it will be seen that the humin N of the feeding-stuffs is very much higher than that of the pure proteins. "Humin" is a name applied to the black substance or mixture of unknown composition which remains undissolved at the conclusion of the hydrolysis of the protein mixture. In pure protein analysis it has been considered to be due to impurities in the sample and to constitute a possible small source of error, through adsorption of small amounts of the amino acids, or possibly, through decomposition of small amounts of the amino-acids through its agency. In the analysis of feeding-stuffs, the carbohydrates,fats and crude fiber present will be found in the humin.

Gortner and Blish have very recently demonstrated that the humin N of pure proteins is increased by the addition of carbohydrates during the hydrolysis. In tests conducted upon pure zein, the humin N of 0.5 gm. was increased from 0.46 percent to 1.84 percent by addition of 0.50 gm. of pure dextrose during the hydrolysis. Similar results have been obtained with gliadin and with

tryptophane. Furthermore they have discovered that when tryptophane is hydrolyzed in the presence of an excess of carbohydrate, 86 percent of its nitrogen is found in the form of humin. Osborne, Van Slyke, Leavenworth and Vinograd, have apparently confirmed this result.[33] Their conclusion, however, that all the humin nitrogen obtained in the analysis of a protein or mixture of proteins is derived from tryptophane can scarcely be supported, however, for Grindley and Slater have shown[34] that lysine and cystine when hydrolyzed in the presence of glucose gives considerable quantities of humin.

It may be pointed out, in this connection, that blood meal, which contains a much higher protein content, and consequently a much lower non-protein content than any of the other feeds examined by Grindley and Slater, gives a smaller result for humin N than any of the other feeding-stuffs. This would seem to support the conclusion that the non-protein substances tend to increase the nitrogen of the humin fraction.

CONCLUSIONS

1. Further confirmation is obtained of the supposition that the Van Slyke method for the analysis of proteins can be applied directly to the analysis of the nitrogen content of feeding-stuffs. Closely agreeing duplicate results are obtained, which must be considered to be approximately accurate.

2. The theory that such analyses of feeding-stuffs will be of practical value in feeding experiments is substantiated by the results obtained for blood meal. This feeding-stuff, which is known to be very valuable as a supplement to other stock foods, is found to contain a relatively high content of lysine, an amino acid whose presence in the food is necessary for the growing animal.

3. The possibility of error in the application of the Van Slyke method to the analysis of feeding-stuffs, due to the presence of non-protein substances in the mixture, is emphasized by the fact that blood meal, which contains less of

these substances than other feeding-stuffs, gives, upon analysis, a smaller percentage of humin nitrogen.

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BIOGRAPHY

The writer was born in Rock Island, Illinois, on December 24th, 1890. He received his early education in the public schools of Rock Island, and in 1911 received the degree of Bachelor of Arts, with honors in Chemistry and Mathematics from Lake Forest College.

During the school years 1911-1912 and 1912-1913, he held the position of Instructor in Mathematics and Science at Manganita Hall, Palo Alto, California.

In 1913, he received an appointment as Graduate Assistant in Chemistry at the University of Illinois, and served in that capacity for two years, while completing the requirements for the degree of Master of Science. During the current year, 1915-1916, he has held the position of Instructor in Chemistry at the State University of Iowa.

The writer is a member of Alpha Chi Sigma and Phi Gamma Delta fraternities.